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1: J Virol. 1991 Nov;65(11):5944-51.

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### Regulated replication of an episomal simian virus 40 origin plasmid in COS7 cells.

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The replication of a simian virus 40 (SV40) origin-containing plasmid, pSLneo, stably transfected COS7 cells has been studied. pSLneo contains the SV40 origin of replication and encodes the positive selection marker for G418 resistance. In transient replication assays, pSLneo replicates to a high copy number in COS7 cells. Uncontrolled SV40 plasmid replication has been reported to be lethal to such transfected cells. Thus, it was anticipated that extensive plasmid replication would preclude isolation of permanent cell lines containing pSLneo. However, significant number of G418-resistant colonies arose after transfection of COS7 cells with pSLneo. Cell lines established from these drug-resistant colonies contained between 1 and 1,000 extrachromosomal pSLneo copies per cell. Episomal plasmid DNA in pSLneo/COS7 lines was stably maintained after 2 months of continuous culture in selective medium. Bromodeoxyuridine labeling and density shift experiments demonstrated that replication of pSLneo closely paralleled that of cellular DNA. On average, plasmid DNA did not replicate more than once during a single cell generation period. Regulation of pSLneo replication appeared to be negatively controlled by a cis-acting mechanism. Endogenous copies of episomal pSLneo remained at a stable low copy number during the simultaneous high-level replication of a newly transfected plasmid encoding SV40 large T antigen in the same cells. These results indicate that regulated replication of an SV40 origin plasmid can be acquired in a cell and not require the presence of additional genetic elements. The molecular mechanism by which cells enforce this regulation on extrachromosomal SV40 plasmids remains to be defined.

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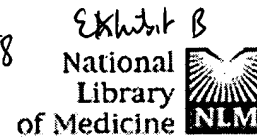
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1: Proc Natl Acad Sci U S A. 1997 Jun 10;94(12):6450-5.

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### Safety-modified episomal vectors for human gene therapy.

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The effectiveness of ongoing gene therapy trials may be limited by the expression characteristics of viral and plasmid-based vectors. To enhance levels of heterologous gene expression, we have developed a safety-modified episomal expression vector that replicates extrachromosomally in human cells. This vector system employs a simian virus 40 (SV40) large T antigen mutant (107/402-T) that is deficient in binding to human tumor suppressor gene products, including p53, retinoblastoma, and p107, yet retains replication competence. These SV40-based episomes replicate to thousands of copies by 2-4 days after gene transfer in multiple types of human cell lines, with lower activity in hamster cells, and no detectable activity in dog and murine cell lines. Importantly, 107/402-T has enhanced replication activity compared with wild-type antigen; this finding may be due, in part, to the inability of p53 and retinoblastoma to inactivate 107/402-T function. We demonstrate that the level and duration of 107/402-T expression regulates the observed episomal copy number per cell. Compared with standard plasmid constructs, episomes encoding 107/402-T yield approximately 10- to 100-fold enhanced levels of gene expression in unselected populations of transiently transfected cells. To determine if 107/402-T-based episomes replicate extrachromosomally in vivo, tumor explants in nude mice were directly injected with liposome/DNA complexes. Using a PCR-based assay, we demonstrate that SV40-based episomes replicate in human cells after direct in vivo gene transfer. These data suggest that safety-modified SV40-based episomes will be effective for cancer gene therapy because high level expression of therapeutic genes in transiently transfected cells should yield enhanced tumor elimination.

#### MeSH Terms:

- Animals
- Antigens, Polyomavirus Transforming/biosynthesis
- Antigens, Polyomavirus Transforming/genetics
- Cell Line
- Comparative Study
- DNA Primers
- DNA Replication
- Dogs
- Gene Therapy/methods\*
- Gene Therapy/standards
- Genes, Tumor Suppressor
- Genetic Vectors\*
- Hamsters
- Human
- Kinetics
- Luciferase/biosynthesis
- Mice
- Polymerase Chain Reaction
- Protein p53/metabolism
- Rats
- Recombinant Proteins/biosynthesis
- Retinoblastoma Protein/metabolism
- Simian virus 40/genetics